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14. ABSTRACT

Indolent prostate cancers that pose very low risk to aged men occur frequently and may be detected at biopsy, leading to the contemporary problem of prostate cancer over-diagnosis and over-treatment. The objective of the project is to define and characterize indolent prostate cancer using genomic approaches in the clinically relevant context of a cohort meeting the entry criteria for active surveillance. During this funding period, we collaborated with urologists and pathologists involved in the active surveillance program, and focused on evaluation of technical feasibility, acquisition of specimen resources, and optimization of the workflow. Our preliminary work established that high quality nucleic acid molecules can be extracted from laser captured small tumor lesions present in men diagnosed with very low risk prostate cancer, setting the stage for large-scale genomic studies.

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Introduction

Indolent prostate cancers that pose very low risk to aged men occur frequently and may be detected at biopsy, leading to the contemporary problem of prostate cancer over-diagnosis and over-treatment. Since progressive acquisition of genomic alterations, both genetic and epigenetic, is a defining feature of all human cancers at different stages of disease progression, RNA and DNA alterations characteristic of indolent prostate tumors may be different from those in clinically significant prostate cancer. However, due to a number of technical constraints, analysis of small volume, very low risk, indolent prostate tumors has not been systemically performed using genome-wide approaches. The primary purpose of the project is to characterize indolent prostate cancer using genomic approaches in the context of a cohort of men predicted to harbor very low-risk prostate cancer at the time of biopsy detection and thus meeting the entry criteria for active surveillance. The scope of the proposed research is: 1) to define the expression signature of indolent prostate cancer by genome-wide expression analysis comparing tissue lesions from very low risk prostate cancer versus high risk prostate cancer defined by pathological outcome measures in men meeting the entry criteria for active surveillance but opting for immediate surgical treatment; 2) to develop a refined signature using biopsy specimens from an active surveillance cohort; and 3) to differentiate indolent prostate cancer from clinically significant prostate cancer using advanced deep-sequencing technologies for both DNA copy number of methylation analysis.

Body

Findings resulting from Task 1: To define indolent human prostate cancer by genome-wide expression analysis comparing tissue lesions from RRP-confirmed very low-risk prostate cancer versus higher-risk prostate cancer (Months 1-24).

Summary: We have completed two critical project milestones associated with Task 1. First we have identified men meeting the active surveillance criteria but that opted for radical retropubic prostatectomy (RRP) treatment, making it possible to perform studies utilizing these pathological specimens representing indolent prostate tumors confirmed by pathological findings. Second we have performed preliminary studies using such specimens, establishing that small-volume tumors present in FFPE sections are compatible with genome-wide RNA analysis.

1. Identification of RRP-confirmed indolent prostate tumors. We performed a survey of surgical prostate cases from men meeting the active surveillance criteria in our institution (i.e., the Epstein criteria) over a 3-month period. The underlying purpose for this survey, assisted by Dr. Epstein (study co-investigator), is to determine whether it is feasible to acquire sufficient number of recently processed FFPE cases that are from men operated for prostate cancer despite meeting the entry criteria for active surveillance. This survey is critical because our previous studies (1, 2) have shown that high-fidelity genomic data can be obtained from these recently processed FFPE specimens. Over a 3-month period, we identified 44 RRP cases fulfilling the pathological criteria for active surveillance. Of these,

21 were organ confined Gleason score 6 consistent with the definition of clinically insignificant (i.e., indolent) prostate tumors, while 23 were upgraded. On the basis of these findings, we expect to have acquired the required number of indolent prostate cancer cases (n=30) during year 2 of the project period for planned genome-wide expression studies.

2. High-quality nucleic acid samples obtained from tissues with indolent prostate cancer. In previous studies, we tackled a number of technical variables relevant to genome-wide expression analysis of formalin fixed paraffin embedded (FFPE) prostate tissue specimens (1, 2). However, our optimized technical procedures had not been tested in small tumors present in FFPE sections from men qualified for active surveillance. We consider a further optimized workflow using target specimens (from men who qualified for active surveillance but opted for surgery) an essential step toward the generation of high-fidelity genomic data. We performed laser-capture microdissection (LCM) (Figure 1, Supporting Data) and downstream RNA extraction (Figure 2, Supporting Data). We show that good quality of RNA sufficient for the proposed studies can be consistently extracted from such cases (Figure 2, Supporting Data).

Findings resulting from Task 2: To validate a refined set of genes predictive or indicative of higher-risk disease within a PAS longitudinal cohort (Months 12-36).

According to our project plan in SOW we will carry out studies related to this task during year 2 and year 3 of the project period and will report relevant findings following the studies.

Findings resulting from Task 3: To define somatic DNA copy number alterations and methylation changes when higher-risk disease develops in men undergoing PAS (Months 1-36).

Summary: According to our project plan in SOW we initially focused on technical optimization and evaluation of the deep sequencing technology, and will carry out DNA copy number and methylation changes in target specimens from men qualified for active surveillance but that opted for surgery during year 2 of the project period.

Technical evaluation using the Illumina HiSeq 2000 platform. To evaluate the various technical aspects of deep sequencing, we subjected DNA samples to X chromosome specific exome capture followed by sequencing using the Illumina HiSeq 2000. The short read sequences (50bp) were aligned by BWA aligner (3). The copy number alterations (CNA) were determined by the following sequential steps: First, the sequence depth was calculated by SAMTools (4); Second, the copy number changes were estimated by comparing the sequence depth to that from normal samples through the VARSCAN software (5); Finally, the copy number segmentations were determined by Circular Binary Segmentation (CBS) algorithm (6). The CNA frequencies of representative tumor samples are shown in Figure 3 (Supporting Data), where red color denotes copy number gain and blue for copy number loss. As shown in Figure 3, the gene AR and OPHN1 had

high frequencies of copy number gain. The region that contains two CT antigen, CT45A4 and CT45A5, had the largest copy number loss. We would like to note that specimens used in this initial evaluation were not from men qualified for active surveillance. Nevertheless, by reliably identifying CNVs in prostate tumor samples using this platform, we gained essential experience with the platform and established the technical feasibility for the proposed studies planned for year 2 of the project period.

Key Research Accomplishments

1. Identified sufficient number of surgical cases from men meeting the entry criteria for active surveillance.
2. Established that high quality nucleic acid molecules can be extracted from laser captured small tumor lesions present in target specimens from men meeting the entry criteria for active surveillance.
3. Optimized the technical steps involved in deep sequencing.

Reportable Outcomes

Manuscripts: None at this time.

Presentations: None at this time.

Grant Applications:

Title: Reducing Prostate Cancer Overdiagnosis and Overtreatment (NIH P01, PI: Pienta)
Supporting Agency: NIH/NCI
Performance Period: 7/1/2014 - 6/30/2019
Level of Funding: $310,000
Role: Project Lead, Project 3
Status: Submitted on Sept. 25th, 2013

Conclusion

High quality nucleic acid molecules can be extracted from laser captured small tumor lesions present in men diagnosed with very low risk prostate cancer. Foreseeable technical barriers presented by large-scale genomic studies of small-volume indolent prostate tumors have been overcome.

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Appendices

None

Supporting Data (3 figures and figure legends)

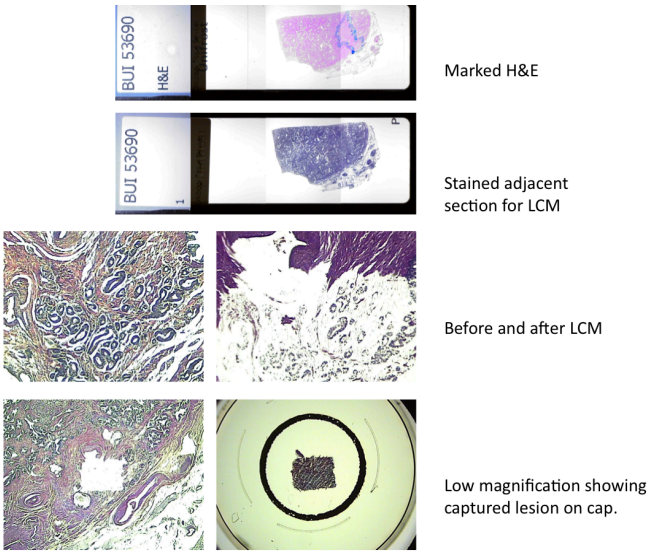

Figure 1: Laser capture microdissection of a marked tumor lesion present in a surgical specimen from a patient meeting the entry criteria for active surveillance.

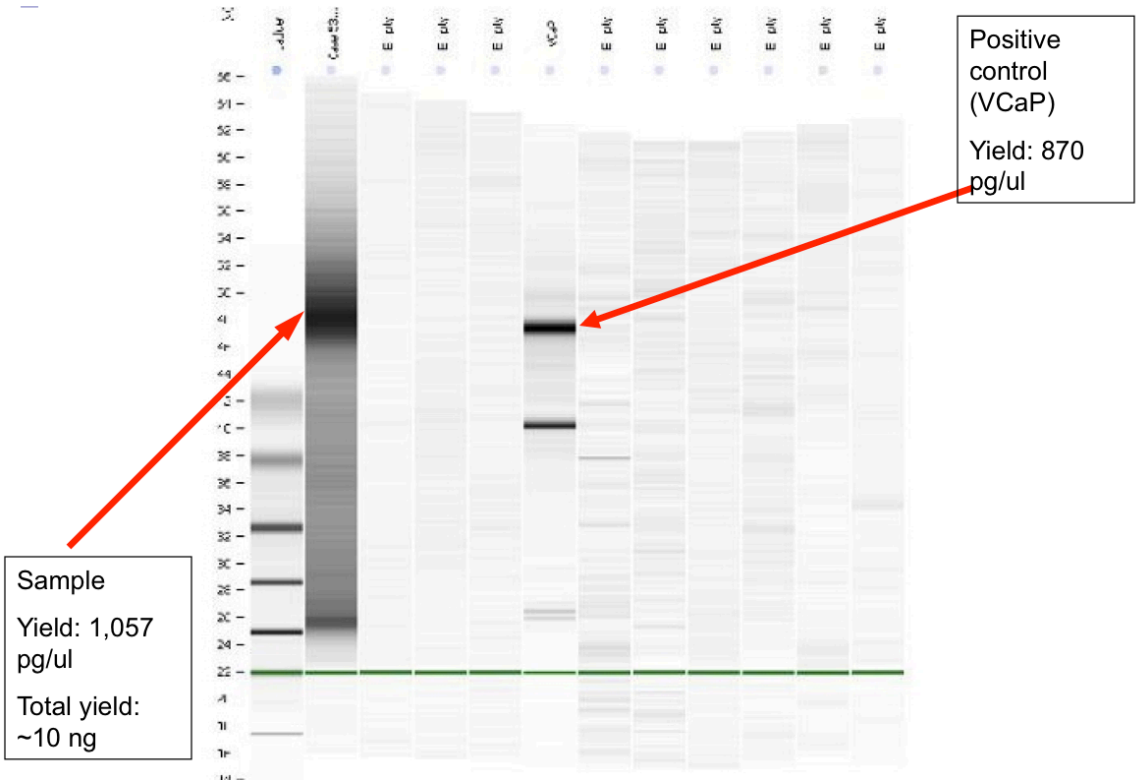

Figure 2: Electropherogram of RNA extracted from laser captured small tumor run on the Agilent Bioanalyzer. The red arrow to the left points to the presence of the 28S rRNA, a marker of RNA quality sufficient for genome-wide RNA analysis. The total yield from the captured lesion is 10ng, also sufficient for the proposed studies. The second red arrow points to a RNA sample prepared from standard cell lines.

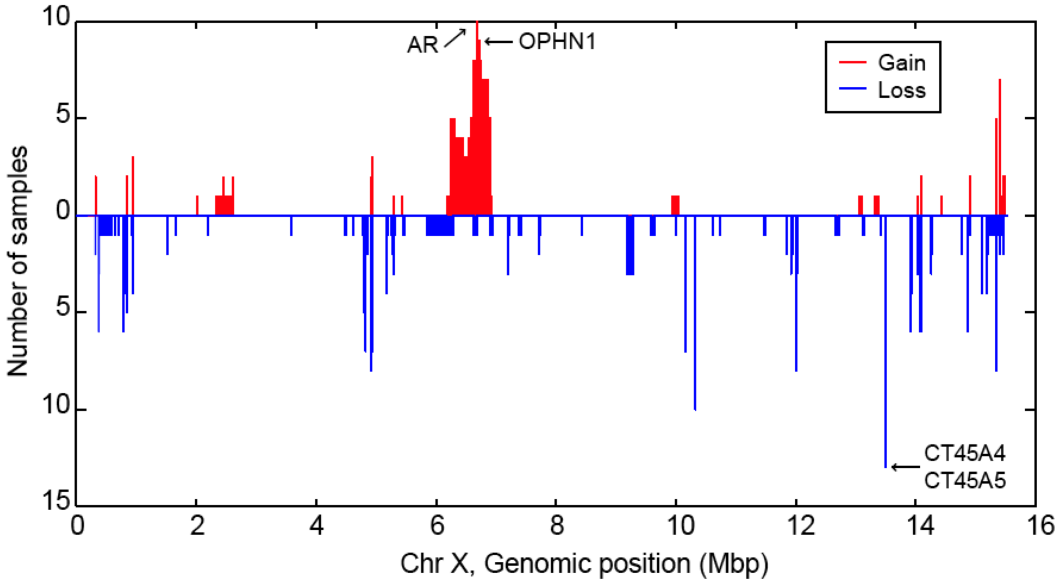

Figure 3. DNA copy number alternations from 14 prostate tumor samples, comparing to copy numbers from 2 normal prostate tissues. Red color denotes copy number gain and blue for copy number loss. The gene *AR* and *OPHN1* had high frequencies, 10 and 9 out of 14 samples, respectively, of copy number gain. The region that contains two CT antigen, *CT45A4* and *CT45A5*, had the largest copy number loss.